METHODS – or INTRODUCTION?

*Study area and sampling:*

Wide coverage, over multiple biomes, varying almost independently in temp and precip (Fig 1a-c)

**1.) Quantifying leaf proteins at the continental scale.** A total of 324 photosynthetically active Eucalypt leaf samples were collected from 32 species; four species were recorded at multiple location. For each species-location combination, three canopy leaves were collected from each of three individuals to make a total of nine samples.

a.) Sampling locations (triangles) were located along three latitudinal bands, spanning broad gradients of rainfall and temperature. The resulting coverage of climate space represents of much of the vegetated area of the Australian continent;

b.) Sampling locations lie within six of the eight biomes described by Whittaker (1967).

c.) Mean annual temperature (oC) and mean annual precipitation (mm, log scaled) of sampling sites (triangles) are distributed orthogonally with respect to one another (r = ).

RESULTS

*Protein composition of the average eucalypt leaf.*

We used the MAPMAN functional annotation scheme to assign proteins to functional categories

In Fig 2a, we show how protein resources are allocated to all major functions in an ‘average’ eucalypt leaf (based on 320 leaf samples).

The majority (64%, SD X%) of protein was associated with photosynthesis; 36% was associated with the carbon fixing Calvin Cycle and 22% (SD X%) with the light reactions (Fig 2a). The most abundant individual protein complexes were Rubisco, comprising 30% (SD X%) of leaf protein and photosystem II (X%, SD X%) (Fig 2b). Protein synthesis, folding and degradation was the second largest top-level category at X% (SD X%) (Fig 2a).

Our mass spectrometry approach allowed detection of X individual proteins per sample, on average. These proteins accounted for 99.9% of sample mass, among which the top 500 most abundant proteins represented 90% (Fig 2c). This is a higher degree of dominance by the top few proteins than observed in [comparison] (Fig 2d), reflecting the specialist nature of leaves as photosynthetic organs.

*Linking leaf protein abundances with environment and functional traits*

Using functionally annotated protein abundance data, we could investigate patterns of protein abundance across environmental gradients, as well as in relation to key leaf functional traits and physiological properties (Fig 2a). Per leaf area protein abundances tended to be strongly cross-correlated.

Positive correlations between leaf nitrogen and all protein functional categories were apparent, as well as somewhat weaker correlations with leaf mass per area. Abundance of proteins associated with individual protein functional categories decline with mean annual temperature and precipitation – a trend which is underpinned by the negative relationship between total leaf protein and these environmental variables (Fig 1e).

~~Proportional protein abundance of a protein functional category indicates investment in a defined function relative to investment in all other functions, and can be viewed as an allocation trait~~. A number of trends in proportional protein abundances (i.e. abundance expressed as a fraction of total protein) were apparent across environmental gradients and in relation to functional traits. For example, allocation to light capturing protein (represented by the ‘photosystems’ category), was negatively related to measures of light availability (incident irradiance and canopy gap fraction). ~~Proportional abundances also offer a clearer means to look at how abundances of proteins associated with different functions are related. For example, protein allocation to photorespiration strongly tracks allocation to Calvin cycle proteins, indicating that greater capacity for carboxylation requires a greater capacity to deal with the consequences of photorespiration.~~

Total leaf protein abundance was strongly driven by temp and to a lesser extent rainfall (Fig 2a, d(i). Individual protein groups are all correlated positively with total protein to varying extent, implicating: a.) a general thermodynamic requirement for greater amounts per leaf area of all major protein functional classes at lower temperatures, and b.) substitution of water use efficiency for N-use efficiency at low rainfall.

*b.) first scatterplot panel*

We selected several relationships for deeper analysis which are of current interest to the vegetation modelling community, but which to date have only been investigated via proxies.

~~We hypothesised that abundance of Calvin cycle proteins would increase with increasing incident solar radiation to maximise photosynthetic capacity, increase with decreasing rainfall so as to maximise CO2 drawdown at low stomatal conductance, and decrease towards warmer sites due decreasing thermodynamic constraints on enzyme-catalysed carbon fixation and associated reactions.~~

We found a strong reduction in Calvin cycle proteins per leaf area in response to MAT (stat, Fig. 2b-i), and to a lesser extent MAP (Fig. 2b-iii), lending support to the hypotheses that Calvin cycle protein abundance is driven by temperature dependence of enzyme kinetics, and maximisation of CO2 drawdown at low stomatal conductance in water-limited environments. Proportional allocation of protein resources to Calvin cycle protein did not adjust over gradients of MAP or MAT (Fig. 2b-ii,iv) but increased marginally (stat) with increasing incident radiation (Fig. 2b-vi). Calvin cycle protein abundance was highly correlated with total protein abundance (Pearson’s r = 0.97), and environmental trends in Calvin cycle protein abundance were essentially identical to trends in leaf protein abundance.

Pronounced declines in both per leaf area and proportional photosystem protein abundance were apparent with incident irradiance (Fig. 2b-v, X% ; (Fig. 2b-vi), X%). No per leaf area response to MAP was observed (Fig. 2b-iii), although proportional abundance of photosystem proteins increased strongly with increasing MAP (Fig. 2b-iv). MAP and incident irradiance are correlated (i.e. denser canopies at wetter sites, Pearson’s r = -0.59) and it is likely that the MAP response of photosystem protein abundance is driven by changing light conditions. Per leaf area photosystem protein abundance was strongly correlated with total leaf protein abundance (Pearson’s r = 0.82) and declined substantially with increasing MAT (Fig. 2b-i).

The range of intraspecific variation in photosystem protein proportional abundance (0.9-0.23, 2.6-fold) was considerably higher than for Calvin cycle proteins (0.30-0.39, 1.3-fold). Thus eucalypt leaves specifically optimised protein allocation to light capture in response to environmental conditions (some stats and numbers), while adjustment of carboxylation capacity was largely achieved through bulk changes in per leaf area protein content.

*c.) second scatterplot panel*

Adjustments in per leaf area Calvin cycle protein abundance occurred to some extent via changes in leaf mass per area (LMA) (Fig. 2c-i). Calvin cycle protein per leaf area increased with increasing LMA, although there was substantial scatter around the Calvin cycle – LMA relationship, indicating that LMA was also responding to requirements other than photosynthetic capacity. Photosystem abundance did not increase per leaf area with increasing LMA (Fig. 2c-ii) and declined as a proportion of total leaf protein. Leaf light harvesting capacity thus appears to be optimised on a per leaf area basis, but independently from leaf thickness.

Leaf nitrogen per area was a strong predictor of both Calvin cycle and photosystem protein abundance per leaf area, and no relative changes in these protein categories occurred with increasing nitrogen per area.

These adjustments are predominantly (though not exclusively) occuring via changes in leaf mass per area and associated increases in leaf nitrogen content. Absolute protein amounts per leaf area are all intercorrelated with each other and also with Amax and LMA and total N per area (Fig 3a lower quadrant, also Fig 3c) – in other words, variation in total protein per leaf area is the dominant influence on variation in amount of any one protein.

How does this section fit in?

*d.) protein abundance multiple regressions*

*The lowest per leaf area protein is associated with low LMA but not low protein %, but high per leaf area protein is associated with high protein % and not high LMA.*

The role of LMA vs protein concentration (i.e. as a fraction of leaf dry mass) in determining per leaf area protein abundance depends interactively on MAP and MAT. Low per leaf area protein abundance at warm, wet sites is more closely associated with low LMA than low protein concentration, while high per leaf area protein abundance at cool, dry sites is strongly associated with high protein concentration. (This isn’t anything that couldn’t have been done using LMA, leaf N% and leaf N\_area, but the point to make is that it’s not all just about increasing carboxylation capacity by adding layers of mesophyll). Wait on though: adding layers of mesophyll (with other cell types kept constant) should increase leaf protein %.

1. Absolute amounts of protein per leaf area adjust along physical gradients as follows (lower row of Fig 2a):
   1. Light reactions decline at higher irradiance but calvin cycle doesn’t change (in other words, at lower irradiance there is more light-capture apparatus relative to CC)
   2. All types decline toward higher temp
   3. toward lower rainfall CC increases, no change to light reactions
   4. each of these make sense for reasonably-well-understood reasons
   5. combined effects in lower row of Fig 3d

No strong effect of environment on proportional allocation of CC’s (although some response to irradiance). Some evidence that carboxylation capacity per leaf area is increased by increasing LMA, although there is substantial variation in the total protein – LMA relationship, indicating that LMA is responding to other requirements than photosynthetic capacity (see last para).